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17 Washing and Sanitizing Treatments for Fruits and Vegetables

Gerald M. Sapers

CONTENTS

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17.1 INTRODUCTION

The detection of human pathogens in fresh produce and occurrence of outbreaks of foodborne illness associated with contaminated produce, as documented in previous chapters, represent serious public health problems. Contamination of fruits and vegetables with human pathogens or organisms causing spoilage also has important economic consequences. Consequently, it is in the interests of the produce industry to develop interventions to reduce the risk of microbial contamination. If contamination is likely during crop production or harvest, it is usually better to reduce this risk by avoidance of contamination sources through implementation of good agricultural practices (GAPs). However, this is not always possible, and in such situations the grower/shipper or processor must depend on washing and sanitizing treatments as a second line of defense. If produce contamination occurs postharvest and contamination sources cannot be eliminated through improvements in plant layout, implementation of good manufacturing practices (GMPs), and improvements in plant sanitation, then washing and sanitizing of produce and equipment become the first line of defense. The subject of washing and sanitizing technology for fresh produce has been reviewed previously [1–3].

In this chapter we review the efficacy, advantages, and disadvantages of conventional washing and sanitizing agents for fresh fruits and vegetables. We also examine the regulatory status of interventions for decontamination of produce and equipment. We examine the types of equipment available for treatment application and their performance. We briefly consider some of the factors that limit the efficacy of cleaning and sanitizing agents and methods of treatment. We examine the potential of new treatments for produce decontamination. We also consider the problem of decontamination of fresh fruits and vegetables in foodservice situations or in the home. This chapter does not examine vapor-phase treatments, surface pasteurization, nonthermal physical treatments, or biological control methods, all of which are covered elsewhere in the book.

17.2 CONVENTIONAL WASHING TECHNOLOGY

17.2.1 Washing Agents

17.2.1.1 Chlorine

Most freshly harvested fruits and vegetables are washed by the grower, packer, or processor to remove soil, plant debris, pesticide residues, and microorganisms from the commodity surface. This may be accomplished by spraying or immersion in water or solutions containing one of a number of cleaning or sanitizing agents, using equipment designed for each particular commodity type, e.g., leafy vegetables, root vegetables, fruit vegetables, tree fruits, or melons. Chlorine is the most widely used sanitizing agent for fresh produce. It may be added to wash water as Cl_2 gas or, more commonly, as sodium or calcium hypochlorite. In water, at pH levels and concentrations used on produce, these chlorine sources are converted to hypochlorous acid and hypochlorite ion in a ratio determined by the solution pH [4,5]. At pH 6.0, roughly 97% of the unreacted chlorine is hypochlorous acid, whereas, at pH 9.0, 97% is hypochlorite ion. The antimicrobial activity of these solutions is due largely to hypochlorous acid rather than to hypochlorite.

The concentration of chlorine in a wash solution is sometimes expressed as total available chlorine (or total residual chlorine = combined residual chlorine + free residual chlorine), based on the calculated amount present in the added hypochlorite or chlorine, or determined by oxidation of KI to I_2, which may not be indicative of the actual potency as a sanitizer because of the inclusion of reaction products such as monochloramine which are not very effective as sanitizers. Preferably, the chlorine concentration can be expressed as free available (or residual) chlorine, the sum of hypochlorous acid and hypochlorite ion concentrations [5]. The total or free chlorine concentration can be monitored by means of test kits, based on colorimetry (www.chemetrics.com; www.emscience.com, www.hach.com), or by measurement of the oxidation–reduction potential (ORP). Chlorine is highly reactive with certain types of compounds in organic materials and soils that are leached or washed from fruits and vegetables. If this chlorine sink is excessive, the free chlorine concentration will be depleted rapidly. Computerized ORP systems that monitor the pH and chlorine concentration can be used to control the level of chlorine in a wash tank in such situations (www.pulseinstruments.net; numerous other suppliers listed on www.globalspec.com).

Use levels of chlorine will depend on allowable levels, the commodity, and the anticipated microbial load. The U.S. Food and Drug Administration (FDA) specifies a use level for washing fruits and vegetables not to exceed 0.2% when followed by a potable water rinse [6]. The U.S. Environmental Protection Agency (EPA) exempts calcium hypochlorite "from the requirement of a tolerance when used preharvest or postharvest in solution on all raw agricultural commodities" [7]. The concentration range of 50 to 200 ppm is commonly used for most commodities. However, as much as 20,000 ppm calcium hypochlorite may be used to sanitize alfalfa seeds intended for sprout production because of the failure of other treatments to disinfect adequately seeds and sprouts, and the high risk that sprouts grown from contaminated seeds may be a source of salmonella or *Escherichia coli* O157:H7 outbreaks [8–10].

Chlorine is highly effective for inactivating planktonic cells of bacteria, yeasts, molds, and viruses, although bacterial and fungal spores are considerably more resistant [5]. However, chlorine is less effective for inactivating bacteria attached to produce surfaces or embedded within the product [11–18]. Typically, population reductions of native microflora on produce surfaces or of human pathogens on inoculated produce are no greater than 2 logs (99%). While such reductions can greatly reduce spoilage, they are insufficient to ensure safety in the event of contamination with human pathogens.

The activity of chlorinated water may be increased by the addition of an acidulant or buffer so that the pH is shifted from an alkaline value (about pH 9) to the neutral to slightly acidic range (pH 6 to 7), thereby increasing the proportion of hypochlorous acid in the equilibrium mixture. Organic acids such as citric acid or mineral acids such as phosphoric or hydrochloric acid can be used for this purpose. If the solution pH is too low (e.g., below pH 4), hypochlorous acid may be converted to free chlorine which is subject to off-gassing. This will result in a loss of activity and may be potentially hazardous. Additionally, equipment corrosion is enhanced as pH levels drop below as well as rise above neutrality. Unpublished data obtained at the Eastern Regional Research Center indicated that hypochlorite solutions acidified with a mineral acid were more stable than solutions acidified with citric acid [19]. Buffers for hypochlorite solutions are available commercially (www.cerexagri.com).

The effectiveness of chlorine in inactivating microorganisms on produce may be enhanced by adding a surfactant to the solution so that it can penetrate into the irregular crevices and pores on produce surfaces where microorganisms may lodge and escape contact with a sanitizer. Several commercial surfactant formulations have been developed for this purpose (www.cerexagri.com/usa/Markets/Cleaners). Addition of a nonionic surfactant improved the efficacy of chlorine against decay fungi in pears [20,21]. Washing formulations containing sodium hypochlorite, buffers, and surfactants have been described by Park *et al.* [22] and marketed by Bonagra Technologies under the name Safe-T-Washed™ (www.bonagra.com). The efficacy of chlorine in reducing the microbial flora of shredded iceberg lettuce was increased by elevating the solution temperature to 47°C [23]. However, no greater reduction of non-pathogenic *E. coli* (ATCC 25922) populations on inoculated apples was obtained when apples were washed at 50 or 60°C compared to 20°C using 200 ppm Cl_2 (added as sodium hypochlorite), adjusted to pH 6.5 with citric acid [19].

Chlorine's major advantages are its broad spectrum of antimicrobial activity, ease of application, and low cost. However, chlorine is highly corrosive and may damage stainless steel equipment after prolonged exposure. Its other major disadvantages are rapid depletion in the presence of a high organic load [24], and the potential carcinogenicity and mutagenicity of its reaction products with organic constituents of foods [25–27]. This is a matter of concern to processors, regulators, and consumers [28]. For these reasons, and the desirability of obtaining greater population reductions, the development of alternative sanitizing agents has been an active area of research, and a limited number of agents suitable for use on fresh produce have been commercialized.

Electrolyzed water, a technology developed largely in Japan [29,30], is really a special case of chlorination. This technology is discussed in detail in Chapter 22.

17.2.1.2 Alternatives to Chlorine

A number of commercial detergent formulations have been developed for washing fruits and vegetables. In addition, three approved sanitizing agents

TABLE 17.1
Advantages and Disadvantages of Commercially Available Sanitizing Agents for Washing Fresh Fruits and Vegetables

Sanitizing agent	Use level (ppm)	Advantages	Disadvantages
Chlorine	50–200	Easy to apply, inexpensive, effective against all microbial forms, not affected by hard water, easy to monitor, FDA approved	Decomposed by organic matter, reaction products may be hazardous, corrosive to metals, irritating to skin, activity pH-dependent, population reductions limited to <1–2 logs
Ozone	0.1–2.5	More potent antimicrobial than chlorine, no chlorinated reaction products formed, economical to operate, self-affirmed GRAS, but FDA review possible, activity not pH-dependent	Requires on-site generation, requires good ventilation, phytotoxic at high concentrations, corrosive to metals, difficult to monitor, higher capital cost than chlorine, no residual effect, population reductions limited to <1–2 logs
Chlorine dioxide	1–5	More potent than chlorine, activity not pH-dependent, fewer chlorinated reaction products formed than with Cl_2, effective against biofilms, FDA approved, residual antimicrobial action, less corrosive than Cl_2 or O_3	Must be generated on-site, explosive at high concentrations, not permitted for cut fruits and vegetables, population reductions limited to <1–2 logs, generating systems expensive
Peroxyacetic acid	≤80	Broad spectrum antimicrobial action, no pH control required, low reactivity with soil, effective against biofilms, FDA approved, no hazardous breakdown products, no on-site generation required, monitoring not difficult, available at safe concentration	Population reduction limited to <1–2 logs, strong oxidant, concentrated solutions may be hazardous

are available as alternatives to chlorine: chlorine dioxide (or acidified sodium chlorite), ozone, and peroxyacetic acid. The advantages and disadvantages of the agents described in the following sections are compared in Table 17.1.

17.2.1.2.1 Detergent Formulations

Among the detergents approved by the FDA for washing produce are sodium *n*-alkylbenzenesulfonate, sodium dodecylbenzenesulfonate, sodium mono- and dimethyl naphthalenesulfonates, sodium 2-ethylhexyl sulfate, and others [6]. These formulations may be neutral in pH, acidic due to the presence of citric or phosphoric acid, or alkaline because of the addition of sodium or potassium hydroxide. Major suppliers of detergent formulations for produce cleaning include Cerexagri (formerly Elf Atochem N.A., Inc., source of Decco products) (800-221-0925; www.cerexagri.com), Microcide, Inc. (www.microcideinc.com), and Alex C. Fergusson, Inc. (800-345-1329; www.afcocare.com).

These products are designed to remove soil and pesticide residues from produce and do not contain antimicrobial agents *per se*. Relatively little information is available concerning the ability of these products to remove or inactivate microorganisms attached to produce surfaces. However, their use can result in significant population reductions. Sapers *et al.* reported that some commercial washing formulations could achieve population reductions as great as 1 to 2 logs in decontaminating apples inoculated with a non-pathogenic *E. coli*, comparable to reductions obtained with hypochlorite [16]. When these products were applied at 50°C instead of at ambient temperature, a 2.5 log reduction was obtained. Wright *et al.* [31] reported similar efficacy with a commercial phosphoric acid fruit wash and with a 200 ppm hypochlorite wash, each applied to apples inoculated with *E. coli* O157:H7. Kenney and Beuchat [32] compared the efficacy of representative commercial cleaning agents in removing or inactivating *E. coli* O157:H7 and *S. muenchen* on spot-inoculated apples. They obtained reductions as great as 3.1 logs with an alkaline product and as great as 2.7 logs with an acidic product, reductions generally being greater with salmonella. Raiden *et al.* [33] compared the efficacy of water, sodium lauryl sulfate, and Tween 80 in removing *Salmonella* spp. and *Shigella* spp. from the surface of inoculated strawberries, tomatoes, and leaf lettuce. They obtained high removal rates but concluded that the detergents were no more effective than water. However, this result may have been a reflection of the brief time interval (1 hour) between inoculation and treatment, which may have been insufficient for strong bacterial attachment. In nature, the interval between preharvest contamination and postharvest application of a wash may be days or weeks, sufficient time for strong attachment and even biofilm formation.

In a study of cantaloupe rind decontamination, Sapers *et al.* [34] reported reductions in the total aerobic plate count of about 1.3 logs when the rind was washed with a 1% solution of a commercial produce wash containing dodecylbenzene sulfonic acid and phosphoric acid (pH 2) at 50°C. Sequential washing with this product followed by treatment with 1% hydrogen peroxide, both at 50°C, resulted in a 3.1 log reduction. Both washes extended the shelf life of fresh-cut cantaloupe prepared from the treated melons. No significant population reductions were obtained when the cantaloupe rind was washed with aqueous solutions of sodium dioctyl sulfosuccinate or sodium 2-ethylhexyl sulfate.

17.2.1.2.2 Chlorine Dioxide

Solutions of chlorine dioxide and acidified sodium chlorite have been used commercially as alternatives to chlorine for sanitizing fresh produce.

Chlorine dioxide is considered to be efficacious against many classes of microorganisms [5]. Chlorine dioxide and acidified sodium chlorite are approved by the FDA for use on fresh produce [35,36], but chlorine dioxide is not permitted for use on fresh-cut products. Chlorine dioxide must be generated on-site, usually by reaction of sodium chlorite with an acid or chlorine gas. Information concerning various proprietary generating and stabilizing systems are available from suppliers such as Vulcan Chemical (800-873-4898), Alcide Corp. (Sanova®; www.alcide.com/sanova), CH2O Inc. (Fresh-Pak™; www.ch2o.com), Rio Linda Chemical Co., Inc. (916-443-4939), Bio-Cide International, Inc. (Oxine®; www.biocide.com), International Dioxcide (www.idiclo2.com), Alex C. Fergusson (800-345-1329; www.afcocare.com), CDG Technology, Inc. (www.cdgtechnology.com), and others. Unlike chlorine, chlorine dioxide is claimed to be effective over a broad range of pH levels, more resistant to neutralization by the organic load, and unlikely to produce trihalomethanes (see Oxine Technical Data Sheet; www.bio-cide.com). Chlorine dioxide also is claimed to be less corrosive than chlorine and to be effective against bacteria in biofilms. However, generation of chlorine dioxide by reaction of sodium chlorite with acid or Cl_2 must be carefully controlled to avoid production of high concentrations of ClO_2 gas which can be toxic and explosive (MSDS for IVR-San 15 sodium chlorite; www.ch2o.com). Additionally, unlike chlorine, chlorine dioxide dissolves in water as a gas and is subject to off-gassing if the water is moving or used in washers. In that situation, special venting would be required to prevent worker discomfort.

The efficacy of chlorine dioxide in disinfecting produce is comparable to that of chlorine. Published reports indicate that chlorine dioxide and related products were potentially effective in preventing potato spoilage by *Erwinia carotovora* [37], reducing populations of *E. coli* O157:H7, *S.* Montevideo, and poliovirus on inoculated strawberries [38], reducing the population of *E. coli* O157:H7 on inoculated apples (but at a treatment level 16 times the recommended concentration) [39], and suppressing decay in pears [40]. Treatments were less effective in suppressing microbial growth on the surface of cucumbers [41]. Fett obtained only a 1 log reduction in alfalfa sprouts irrigated with acidified sodium chlorite [42]. Population reductions of *L. monocytogenes* on uninjured surfaces of inoculated green bell peppers, washed with ClO_2 solution (3 mg/l), were about 2 logs greater than could be achieved with a water wash, but reductions were negligible on injured surfaces [43]. In contrast, these investigators obtained population reductions of 7.4 and 3.6 logs on uninjured and injured surfaces of peppers, respectively, using a ClO_2 gas treatment (see Chapter 18).

17.2.1.2.3 Ozone

The efficacy of ozone in killing human pathogens and other microorganisms in water is well established [44], and it is widely used as an alternative to chlorine in municipal water treatment systems and for production of bottled water

[45]. Ozone is effective in killing food-related microorganisms [46] and has been approved for use on foods by the FDA [47]. Potential applications of ozone in disinfecting foods have been reviewed [48,49]. Ozone is effective in reducing bacterial populations in flume and wash water and may have some applications as a chlorine replacement in reducing microbial populations on produce [50,51]. Ozone treatment was effective in suppressing decay of table grapes by *Rhizopus stolonifer* [52]. Use levels of 0.5 to 4.0 μg/ml are recommended for wash water and 0.1 μg/ml for flume water [53,54].

However, not all ozone treatments show high efficacy. Ozone treatment of fresh-cut lettuce, inoculated with a mixture of natural microflora, yielded reductions of only 1.1 logs [18]. Treatment of lettuce, inoculated with *Pseudomonas fluorescens*, with 10 μg/ml of ozone for 1 minute achieved less than a 1 log population reduction [50]. While ozone treatment of apples inoculated with *E. coli* O157:H7 was effective in reducing populations on the surface (3.7 log reduction), reductions were <1 log in the stem and calyx regions [55]. Ozone treatment of pears (5.5 μg/ml water for 5 minutes) was ineffective in reducing postharvest fungal decay [56]. Population reductions obtained by ozone treatment of alfalfa seeds inoculated with *E. coli* O157:H7 were only marginally better than those for water-treated controls [57]. In another study, ozone treatment of alfalfa seeds, inoculated with *L. monocytogenes*, was ineffective in reducing the population of this pathogen, while treatment of inoculated alfalfa sprouts reduced the *L. monocytogenes* population by <1 log and was phytotoxic to the sprouts [58]. These results are probably a reflection of the difficulty in contacting and inactivating bacteria attached to produce surfaces in inaccessible sites (see Chapters 2 and 3).

One of the major advantages claimed for ozone is the absence of potentially toxic reaction products. However, ozone must be adequately vented to avoid worker exposure [48]. Ozone has to be generated on-site by passing air or oxygen through a corona discharge or UV light [48]. A number of commercial systems for generating ozonated water for produce washing are available. Information about commercial ozone generators is available on-line from Air Liquide (www.airliquide.com), Praxair, Inc. (www.praxair.com), Novazone (www.novazone.net), Pure Ox (www.pureox.com), Osmonics, Inc. (www.osmonics.com/food), Ozonia North America, Inc. (www.ozonia.com), Lynntech, Inc. (www.lynntech.com), Clean Air & Water Systems, Inc. (360-394-1525), Electric Power Research Institute (EPRI; www.epri.com), and others. For information about ozone gas disinfection treatments, see Chapter 18.

17.2.1.2.4 Peroxyacetic Acid

Peroxyacetic acid (peracetic acid) is an equilibrium mixture of the peroxy compound, hydrogen peroxide, and acetic acid [59–61]. The superior antimicrobial properties of peroxyacetic acid are well known [59]. Peroxyacetic acid is approved by the FDA for addition to wash water at concentrations not to exceed 80 ppm [6]. Under EPA regulations, an exemption from the requirements of a tolerance was established for peroxyacetic acid as an antimicrobial treatment for fruits and vegetables at concentrations up to 100 ppm [62]. Much higher concentrations are permitted for sanitizing food contact surfaces [63]. Peroxyacetic acid decomposes into acetic acid, water, and oxygen, all harmless residuals.

Peroxyacetic acid is recommended for use in treating process water, but Ecolab, one of the major suppliers, is also claiming substantial reductions in microbial populations on fruit and vegetable surfaces [64]. However, company literature provides insufficient information on methodology to assess treatment efficacy (www.ecolab.com/initiatives/foodsafety). Population reductions for aerobic bacteria, coliform bacteria, and yeasts and molds on fresh-cut celery, cabbage, and potatoes treated with 80 ppm peroxyacetic acid were less than 1.5 logs [65]. Addition of 40 ppm Tsunami 100 (the Ecolab peroxyacetic acid product) to the irrigation water used during sprout propagation did not suppress the outgrowth of the native microflora [42]. Treatment with 100 ppm Tsunami reduced the population of *E. coli* O157:H7 and *S.* Montevideo on inoculated strawberries by about 97% [38]. Several published studies have looked at the efficacy of peroxyacetic acid against *E. coli* O157:H7 on inoculated apples. Attempts to disinfect apples, inoculated with *E. coli* O157:H7, by washing with 80 ppm peroxyacetic acid 30 minutes after inoculation resulted in a 2 log reduction compared to a water wash [31]. However, in another study where inoculated apples were held for 24 hours before washing (allowing more time for attachment), an 80 ppm peroxyacetic acid treatment reduced the *E. coli* O157:H7 population by less than 1 log; at 16 times the recommended concentration, a 3 log reduction was obtained [39]. Sapers *et al.* [16] reported similar results with apples inoculated with a nonpathogenic *E. coli*. Like ozone and chlorine dioxide, low concentrations of peroxyacetic acid are effective in killing pathogenic bacteria in aqueous suspension [59]. Addition of octanoic acid to peroxyacetic acid solutions increased efficacy in killing yeasts and molds in fresh-cut vegetable process waters but had little effect on population reductions on fresh-cut vegetables [65].

Peroxyacetic acid is a strong oxidizing agent and can be hazardous to handle at high concentrations, but not at strengths marketed to the produce industry. Peroxyacetic acid is available at various strengths from Ecolab, Inc. (www.ecolab.com), FMC Corp. (www.fmcchemicals.com), and Solvay Interox (www.solvayinterox.com).

17.2.2 Washing Equipment

17.2.2.1 Types of Washers

Washing equipment for produce is designed primarily for removal of soil, debris, and any pesticide residues from the harvested commodity. The design of most commercial equipment has not taken into account requirements for the reduction of microbial populations on produce surfaces although this is a desirable goal of washing.

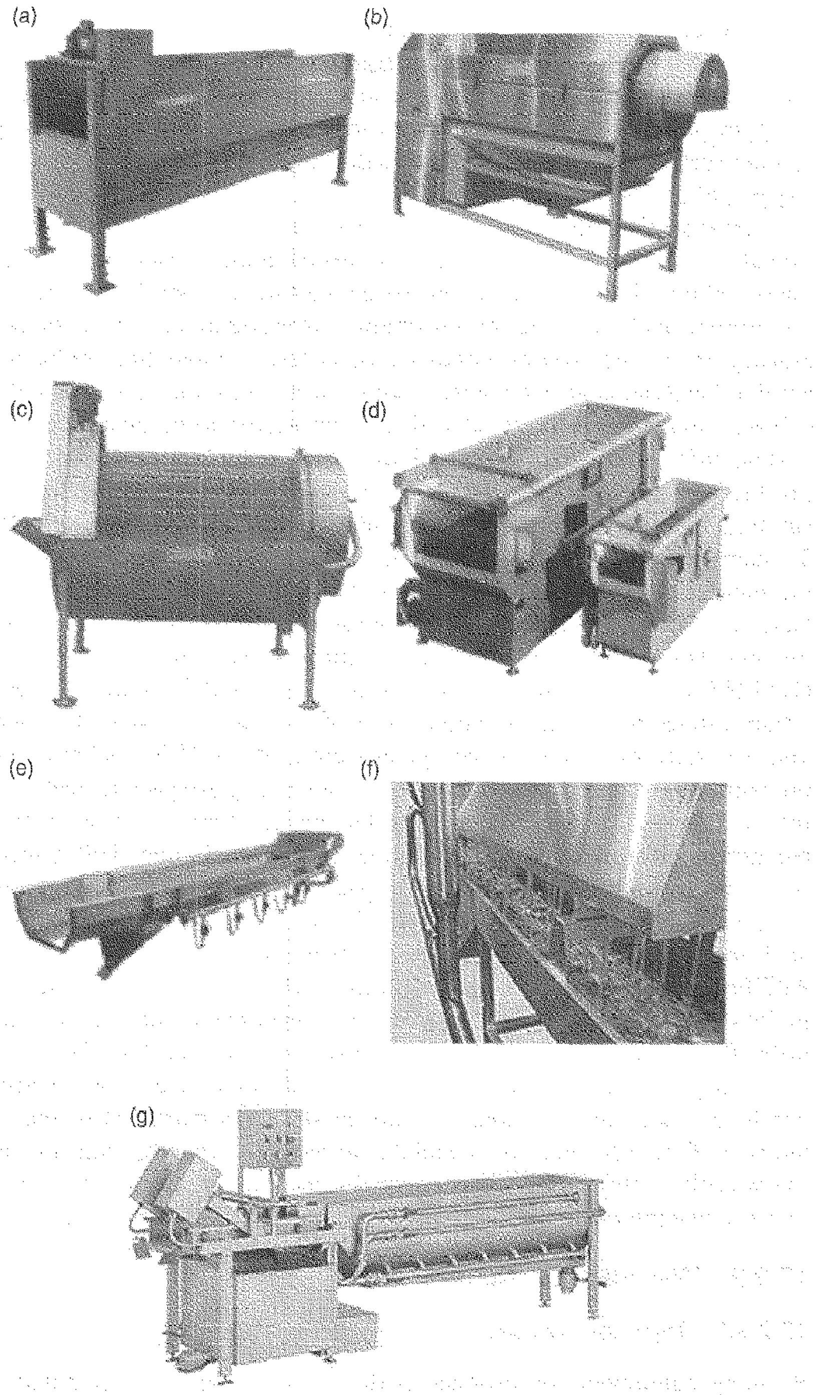

FIGURE 17.1 Commercial washing equipment for fruits and vegetables: (a) flat-bed brush washer; (b) U-bed brush washer; (c) rotary washer; (d) pressure washer; (e, f) flume washers; (g) helical washer.

Numerous types of washers have been developed for cleaning fresh fruits and vegetables, varying in complexity from a garden hose used for cleaning apples prior to farm-scale cider production (an unsatisfactory procedure due to lack of control) to sophisticated systems employing rotating brushes and applying heated water under pressure with agitation. The more common types of commercial washers for produce include dump tanks, brush washers, reel washers, pressure washers, hydro air agitation wash tanks, and immersion pipeline washers (Figure 17.1). Major suppliers of such equipment are listed on the Postharvest Resources website of the University of Florida (http://postharvest.ifas.ufl.edu). The choice of washer for a particular commodity will depend on such characteristics of the commodity as shape, size, and fragility. It is obvious that equipment requirements are quite different for cut lettuce than for tomatoes or potatoes.

17.2.2.2 Efficacy of Washers

The efficacy of commercial flat-bed and U-bed brush washers in removing or inactivating a nonpathogenic *E. coli* on artificially contaminated apples was investigated by Annous *et al.* [66] and Sapers [3]. These studies demonstrated that the *E. coli* population could be reduced by about 1 log (90%) by passage of the apples through a dump tank with minimal agitation (Table 17.2). However, further cleaning of the apples in a flat-bed brush washer had little further effect on the *E. coli* population, irrespective of the cleaning or sanitizing agent used (water, 200 ppm Cl_2, 1% acidic detergent, 8% trisodium phosphate, 5% H_2O_2). Similar results were obtained with a U-bed brush washer. Subsequent studies by the investigators showed that the bacteria that had attached in the

TABLE 17.2
Decontamination of Apples Inoculated with *E. coli* (Strain K12) with Sanitizing Washes Applied in a Flat-Bed Brush Washer

		E. coli ($\log_{10}$ CFU/g)[a]		
Wash treatment	Temp. (°C)	Before dump tank	After dump tank	After brush washer
Water	20	5.49 ± 0.09	4.92 ± 0.37	4.81 ± 0.26
	50	5.49 ± 0.09	5.03 ± 0.15	4.59 ± 0.08
200 ppm Cl_2	20	5.87 ± 0.07	5.45 ± 0.05	5.64 ± 0.23
8% Na_3PO_4	20	5.49 ± 0.09	5.02 ± 0.43	4.98 ± 0.02
	50	5.49 ± 0.09	5.02 ± 0.08	4.75 ± 0.45
1% acidic detergent	50	5.87 ± 0.07	5.49 ± 0.03	5.42 ± 0.50
5% H_2O_2	20	5.87 ± 0.07	5.46 ± 0.40	5.27 ± 0.09
	50	5.87 ± 0.07	5.54 ± 0.31	5.49 ± 0.10

[a] Mean of four determinations ± standard deviation.

From Annous, B.A. *et al.*, *J. Food Prot.*, 64, 159, 2001. Reprinted with permission. Copyright International Association for Food Protection, Des Moines, IA.

TABLE 17.3
Distribution of *E. coli* (ATCC 25922) on Surfaces of Inoculated Apples Before and After Washing with 5% H_2O_2 at 50°C

	Log_{10} (CFU/cm^2)			
	24 h after inoculation		72 h after inoculation	
Location	Inoculated	Washed	Inoculated	Washed
Skin except at calyx and stem ends	4.77	2.05	4.37	1.63
Skin at calyx end of core	7.26	5.20	6.79	4.46
Skin on stem end of core	6.63	5.06	5.61	4.89

From Sapers, G.M. *et al.*, *J. Food Sci.*, 65, 529, 2000. Reprinted with permission.

relatively inaccessible stem and blossom ends of the apples, or were internalized within the latter region, survived washing while *E. coli* attached elsewhere on the apple surface were readily inactivated (Table 17.3). Greater efficacy was obtained when the apples were washed by full immersion in a sanitizing solution with vigorous agitation [67].

Gagliardi *et al.* [68] examined commercial practices for washing melons produced in the Rio Grande River Valley of Texas. They reported little or no reduction in the population of coliforms, fecal coliforms, enterococci, and fecal enterococci in cantaloupes and honeydew melons that were washed with water in a tank and then spray rinsed on a conveyor line. Use of chlorinated water in the secondary rinse appeared to reduce the populations of fecal coliforms and fecal enterococci but not total coliforms and enterococci. Laboratory-scale washing studies with cantaloupes that had been dip-inoculated with *Salmonella* Stanley or a nonpathogenic *E. coli* (ATCC 25922) demonstrated that the population reductions obtained by immersion of the melons in 200 ppm Cl_2 or 5% H_2O_2 decreased as the time interval between inoculation and washing increased from 24 hours to 5 days [69,70]. However, the efficacy of these treatments in inactivating *L. monocytogenes* on inoculated cantaloupes was not dependent on the length of storage between inoculation and treatment [71]. Sapers *et al.* obtained minimal inactivation of *E. coli* B-766 (a surrogate for *S.* Poona) when dip-inoculated cantaloupes were immersed in 300 ppm Cl_2 for 3 minutes [72]. Apparently, cantaloupes are especially difficult to disinfect, even if fully immersed in the sanitizing solution. This may be due to the movement, attachment, and possible biofilm formation by the targeted bacteria within inaccessible pores in the netting so that contact between the sanitizing solution and the attached bacteria is minimal. This is borne out by the success of treatments with 5% H_2O_2 at 70°C or near boiling water where heat penetration contributes to the efficacy of the antimicrobial treatment [73] (see Chapter 10). Such treatments can greatly reduce the risk of transfer of human pathogens from the rind surface to the flesh during fresh-cut processing.

17.2.3 Factors Limiting the Efficacy of Washing

The action of commercial washing agents and equipment in removing or inactivating microorganisms on fresh produce is not well understood. In general, microbial populations on produce surfaces are not easily detached or inactivated for a number of reasons discussed in Chapters 2 and 3. Briefly, the microbial contaminants may become strongly attached to the produce surface by physical forces within a short time of contamination or incorporated within a biofilm over a longer time period. Microbial contaminants may be located in a protected attachment site, e.g., a cut surface, puncture, or pore, where a wash solution cannot reach. Microorganisms also may become internalized within the commodity either during crop production or when submerged in water in a packing plant dump tank or flume as a consequence of infiltration driven by a negative temperature differential or by hydrostatic pressure. Consequently, the inaccessible population will escape direct contact with a cleaning or sanitizing agent in a commercial washer. These conditions are discussed in greater detail in an earlier review article [3] and in Chapter 3.

17.3 NOVEL WASHING TECHNOLOGY

Because the commercially available alternatives to chlorine discussed above generally cannot achieve population reductions of human pathogens on contaminated produce much in excess of 2 logs, which is insufficient to ensure safety, a number of experimental treatments have been examined to obtain greater efficacy. The efficacy and regulatory status of some of these experimental treatments are described in the following.

17.3.1 Hydrogen Peroxide

Hydrogen peroxide is a highly effective antimicrobial agent against bacteria but is less active against yeasts, fungi, and viruses [59]. Characteristics and potential food applications of hydrogen peroxide as a sanitizer for produce were recently reviewed by the author [74]. Hydrogen peroxide may be considered as a potential alternative to chlorine. Numerous studies have demonstrated the efficacy of dilute hydrogen peroxide in sanitizing fresh produce including mushrooms [75–77], apples [16,67,78], melons [34,69,70,73], eggplant, and sweet red pepper [80]. In side-by-side comparisons, dilute (1 to 5%) hydrogen peroxide washes were at least as effective as 200 ppm chlorine [16,79]. When applied to apples with vigorous agitation at an elevated temperature (50 to 60°C), population reductions approaching 3 logs were obtained [67]. However, temperatures exceeding 60°C could not be used without inducing browning of the apple skin. Hydrogen peroxide treatments were ineffective in decontaminating sprouts [42] or the seeds used to produce sprouts [81].

While treatment with hydrogen peroxide vapor can reduce microbial populations on grapes [82], melons [83], and prunes [84], required treatment times are long compared to the application of a dilute hydrogen peroxide dip [85]. The vapor treatments proved to be ineffective with apples [86] and produced discolorations with mechanically damaged berries [85].

The regulatory status of hydrogen peroxide as a washing agent for produce is unclear. The FDA has jurisdiction if the washing treatment is applied as part of a processing operation, while the EPA has jurisdiction if the treatment is applied to a raw commodity. While fresh produce clearly falls within the EPA regulations, fresh-cut produce is under FDA regulations. However, if the wash treatment is applied to the raw produce before cutting, and if this operation is carried out in a receiving area, separate from the processing room, it would appear that EPA regulations apply. Under FDA regulations, hydrogen peroxide is GRAS (generally recognized as safe) for some specified food applications, provided that residual H_2O_2 is removed "by appropriate physical and chemical means during processing," but the regulation does not cover hydrogen peroxide as a washing or sanitizing agent for produce [87]. According to an Agency Response Letter (GRAS notice no. GRN 000014, May 26, 1999) a petition to the FDA to amend the regulation would be required to seek approval for a new application (in this case, reduction of the microbial load on onions prior to dehydration; http://vm.cfsan.fda.gov/~rdb). Peroxyacetic acid formulations, which contain low levels of hydrogen peroxide (59 ppm), are approved by the FDA for use in washing fruits and vegetables [6]. A higher concentration is permitted if the formulation is used to sanitize food contact surfaces [63] Under EPA regulations, postharvest hydrogen peroxide applications to produce as an antimicrobial treatment are exempt from the requirements of a tolerance if the concentration is $\leq 1\%$ per application [88].

The presence of residual hydrogen peroxide should not represent an obstacle to use of this agent as a produce sanitizer. Most fruits and vegetables contain sufficient catalase to permit rapid breakdown of residual peroxide to water and oxygen. Peroxide residues could not be detected in mushrooms, apples, or cantaloupes following hydrogen peroxide wash treatments [16,34,77].

Information on hydrogen peroxide applications can be obtained from FMC Corp. (www.fmcchemicals.com), Solvay Interox (www.solvayinterox.com), US Peroxide (h2o2.com), and Degussa Corp. (www.degussa.com). BiosSafe Systems (www.biosafesystems.com) is marketing a formulation containing hydrogen peroxide and peroxyacetic acids (Storox®) for sanitizing fruits and vegetables; the recommended maximum use level is 0.27%.

17.3.2 Trisodium Phosphate and Other Alkaline Washing Agents

Trisodium phosphate (TSP) has been marketed by Rhodia Specialty Phosphates (www.rhodia-phosphates.com) as an antimicrobial rinse (AvGard®, Assur-Rinse®) to reduce human pathogen populations on processed beef and poultry. TSP is classified as GRAS by the FDA [89].

The antimicrobial activity of TSP probably is due to its high pH (pH 12) which disrupts the cytoplasmic membrane [90,91]. Highly alkaline washes based on sodium and potassium hydroxide (pH 11 to 12) resulted in 3 log reductions in the population of a nonpathogenic *E. coli* on surface-inoculated oranges [92]. A 30-minute dip in 0.25% calcinated calcium suspension, another highly alkaline product derived from oyster shells (pH 10), reduced the native bacterial population on cucumbers by about 2 logs [93]. In a more recent study, Bari *et al.* [94] reported population reductions exceeding 5 logs on tomatoes that had been surface inoculated with *E. coli* O157:H7, salmonella strains, or *L. monocytogenes* and treated with 0.5% calcinated calcium. These exceptionally high population reductions (for a wash) may be a reflection of the brief interval (30 minutes) between inoculation and treatment used by these investigators. Sapers *et al.* [67] obtained population reductions approaching 3 logs when apples that had been dip-inoculated with *E. coli* (ATCC 25922) were washed with 5% hydrogen peroxide, followed by brushing the calyx and stem areas with a paste of calcinated calcium; the population reduction was <2 logs with only the peroxide wash. TSP solutions (12 to 15%) were highly effective in reducing *S.* Montevideo populations on inoculated tomato surface but failed to inactivate completely this organism in the tomato core tissue [95]. Survival in the latter tissue probably resulted from bacterial infiltration. Sapers *et al.* [78] reported a 2 log reduction in a nonpathogenic *E. coli* strain on inoculated apples washed with 4% TSP at 50°C. A 1% TSP wash reduced the population of *E. coli* O157:H7 and *S.* Montevideo on strawberries by 93 and 96%, respectively [38]. Treatment of lettuce with 2% TSP was ineffective in killing *L. monocytogenes* [14]. Addition of 0.3% TSP to the irrigation water was ineffective in reducing the native microflora on alfalfa sprouts [42]. TSP was reported to be highly effective in inactivating *E. coli* O157:H7 in biofilms but less effective against *S.* Typhimurium and *L. monocytogenes* in biofilms [96].

17.3.3 Organic Acids

Organic acids such as lactic and acetic acids are effective antibacterial agents [97] and are classified by the FDA as GRAS [98,99] (21CFR184.1005; 21CFR184.1061). Lactic acid dips and sprays are used commercially to decontaminate animal carcasses containing *E. coli* O157:H7, *L. monocytogenes*, and salmonella [100] (see additional information from Purac America, Inc., www.purac.com). Lactic acid rinses might have applications for the decontamination of fruits and vegetables. A 5% acetic acid wash was reported to reduce the population of *E. coli* O157:H7 on inoculated apples by about 3 logs [31]. In another study, apples that had been inoculated with *E. coli* O157:H7 were treated with 5% acetic acid at 55°C for as long as 25 minutes. While the *E. coli* population was greatly reduced in the apple skin and stem areas, as many as 3 to 4 logs survived in the calyx tissue [101]. In a more recent study, application of 2.4% acetic acid to apple disks that had been inoculated with *S. mbandaka* or *S.* Typhimurium resulted in population reductions of 1.1 and 1.4, respectively [102]. However, the combination of 5% acetic acid with 5% hydrogen peroxide yielded a population reduction approaching 4 logs. It is not clear whether organic acid treatments would produce off-flavors or discoloration in treated produce.

17.3.4 Other Experimental Antimicrobial Washing Agents

Cetylpyridinium chloride (CPC) is being marketed as Cecure® for use in oral hygiene products and may have application as an antimicrobial rinse for fresh produce and other foods. Yang *et al.* [103] reported population reductions in the range 1 to 2 logs for *S.* Typhimurium and *E. coli* O157:H7 on inoculated fresh-cut lettuce, treated by spraying with 0.3% CPC. Similar reductions were obtained with strawberries inoculated with *E. coli* O157:H7 or *S.* Montevideo and immersed in 0.1% CPC at 43°C [38]. However, regulatory approval for this agent has not yet been obtained (www.safefoods.net/cecure.htm). Activated lactoferrin, which prevents attachment of bacteria to meat, is approved by the FDA and USDA for application to beef as a carcass rinse [104] (also see www.activinlf.com). However, there are no reports of its applicability to fruits and vegetables. Silver and copper ions are known to exert antimicrobial activity against bacteria in water [105], and ion generators have been marketed for disinfection of water in swimming pools, irrigation systems, and various other commercial applications (Tew Manufacturing Corp., 800-380-5839; T.P. Technology plc, www.tarn-pure.com). Application of this technology to produce packing lines and dump tanks at recommended levels of 0.50 ppm copper and 0.035 to 0.05 ppm silver has been proposed (Tew Manufacturing Corp.), but published efficacy data are lacking, and the regulatory status of such applications is unclear.

17.3.5 Synergistic Treatment Combinations

Certain combinations or sequences of treatments may show synergism in inactivating or detaching microbial contaminants on produce. Such behavior might be anticipated if the individual treatments have different modes of action, e.g., cell membrane disruption and oxidation. Several examples of promising combination treatments have been reported: the sequential washing of cantaloupes with detergents and hydrogen peroxide [34] and the application of an acetic acid–hydrogen peroxide combination to inoculated apple disks [102]. Lin *et al.* [106] investigated the inactivation of *E. coli* O157:H7, *S. enterica* serotype Enteritidis, and *L. monocytogenes* by combinations of hydrogen peroxide and lactic acid and hydrogen peroxide with mild heat. Further research in this area may yield treatment combinations that show greater efficacy towards bacteria located in punctures or pores or incorporated in biofilms on produce surfaces.

17.4 FOODSERVICE AND HOME APPLICATIONS

While conventional sanitizing agents, applied to produce with commercial-scale washing equipment, have the capability of achieving 1 to 2 log population reductions in contaminated produce, this option is not generally available for foodservice and consumer applications. Consumers and operators of delicatessens, restaurants, and other foodservice establishments do not have the technical skills or knowledge to prepare the more potent sanitizer solutions used commercially nor do they have access to commercial washing equipment. Duff *et al.* [107] developed an economic model to evaluate the potential cost-effectiveness of a disinfection program that targets high-risk food preparation activities in household kitchens. They concluded that such a program would be cost-effective. What options are available to consumers and foodservice managers so that they can provide some meaningful level of protection to their families or customers?

17.4.1 FDA Recommendations

The FDA advises consumers to: "Wash all fresh fruits and vegetables with cool tap water immediately before eating. Don't use soap or detergents. Scrub firm produce, such as melons and cucumbers, with a clean produce brush. Cut away any bruised or damaged areas before eating." Consumers are also advised to:

> Wash surfaces often. Cutting boards, dishes, utensils, and counter tops should be washed with hot soapy water and sanitized after coming in contact with fresh produce, or raw meat, poultry, or seafood. Sanitize after use with a solution of 1 teaspoon of chlorine bleach in 1 quart of water. Don't cross contaminate. Use clean cutting boards and utensils when handling fresh produce. If possible, use one clean cutting board for fresh produce and a separate one for raw meat, poultry, and seafood. During food preparation, wash cutting boards, utensils, or dishes that have come into contact with fresh produce, raw meat, poultry, or seafood. Do not consume ice that has come in contact with fresh produce or other raw products (www.fda.gov/bbs/topics/ANSWERS/2002/ANS01167.html/).

In the situation where a particular fruit or vegetable is suspect, more specific advice is provided. For example, in response to an outbreak of hepatitis A in green onions, the FDA recommended: "Cook green onions thoroughly. This minimizes the risk of illness by reducing or eliminating the virus. Cook in a casserole or sauté in a skillet" and "Cook sprouts. This significantly reduces the risk of illness" [108]. While a kill step is undoubtedly effective, it would not be applicable to many fruits and vegetables that would no longer be considered "fresh" if subjected to a cook or full blanch and would lose their appeal to consumers. Washing produce without a sanitizer is not likely to achieve the population reductions that can be obtained with commercial sanitizing agents and equipment.

17.4.2 Other Options

Alternative methods of surface sanitizing cantaloupes were examined by Barak *et al.* [109]. They reported reductions in the bacterial load of 70, 80, and 90% by scrubbing the melons with a vegetable brush in tap water, washing with soap, and dipping in 150 ppm sodium hypochlorite, respectively. However, a three-compartment sanitation method comprising washing with an antimicrobial soap, scrubbing with a brush in tap water, and immersion in a hypochlorite solution resulted in a 99.8% reduction. Population reductions exceeding 5 logs were obtained on cut iceberg lettuce, inoculated with *E. coli* CDC1932, by washing with diluted vinegar (1.9% acetic acid); in contrast, washing with diluted bleach solution (180 ppm available chlorine) and lemon juice (0.6% citric acid) yielded 1.6 and 2.1 log reductions, respectively [110]. However, the vinegar treatment resulted in some product damage. Application of a solution containing 1.5% lactic acid and 1.5% hydrogen peroxide as a 15-minute soak at 40°C was reported to yield greater than 5 log reductions in the population of *E. coli* O157:H7, *Salmonella enteritidis*, and *Listeria monocytogenes* on spot-inoculated apples, oranges, and tomatoes [111]. However, in both studies, the surviving bacteria were recovered by a rinsing procedure such that only unattached, exposed cells were being recovered and not bacteria that were embedded in fruit tissues or biofilms or attached to fruit surfaces. This may have yielded unrealistically high population reductions. Smith *et al.* [112] evaluated a commercial peroxyacetic acid formulation intended for foodservice applications (Victory produce wash; Ecolab, St. Paul, MN; www.ecolab.com) for reducing the bacterial load on lettuce; small reductions (~1 log) were obtained. Lukasik *et al.* [38] compared various washing treatments, including consumer-oriented products (detergents, Fit® and Healthy Harvest) on inoculated strawberries; population reductions for *E. coli* O157:H7, *S. montevideo*, and several viruses were between 1 and 2 logs. Parnell and Harris [113] compared water, sodium hypochlorite, and vinegar as consumer washes for reducing salmonella on spot-inoculated apples. Population reductions obtained with vinegar and chlorine washes were 2 to 3 logs greater than reductions obtained with water. Treatment with sodium hypochlorite and vinegar yielded comparable reductions in the population of natural microbiota of lettuce [114]. A study of consumer acceptance of a home use antibacterial solution for sanitizing apples indicated that consumers would be unwilling to use a procedure requiring the 15-minute heat and soak step [115]. Venkitanarayanan *et al.* [116] reported that an electrolyzed water treatment was effective in inactivating foodborne pathogens on smooth plastic kitchen cutting boards. They did not investigate scarred cutting boards which might be expected in a kitchen or foodservice situation.

17.4.3 Commercial Equipment and Wash Formulations for Home or Foodservice Use

Some manufacturers of commercial equipment for sanitizing produce have developed small-scale units suitable for consumer and foodservice use. Systems based on use of electrolyzed water are being marketed by Sterilox Technologies, Inc. (www.steriloxtechnologies.com) and Hoshizaki America, Inc. (www.hoshizakiamerica.com). Small-scale systems based on ozone are being marketed by Sterilion Ltd (www.performancesystems.com/medical.htm) and UltrOzone (UC Davis Postharvest Technology Center; 1-866-21-OZONE).

A number of commercial fruit and vegetable wash formulations intended for consumer use are being marketed, but little information is available about their performance in reducing microbial populations. Fit®, a produce wash produced by Procter & Gamble Co. and marketed for a number of years, did show some antimicrobial activity in addition to removing dirt, wax, and other residues [117,118], although no claims were made by the company that the consumer product had antimicrobial activity. They did make such a claim for a "Pro Line Fit" intended for commercial rather than consumer use. Fit is now marketed by HealthPro Brands, Inc. (www.healthprobrands.com). JohnsonDiversey markets a Hard Surface Sanitizer/Fruit & Vegetable Wash (Product 4444) claimed to have antimicrobial activity (www.jwp.com/jwp/ProdInfo.nsf/; click on foodservice, then sanitizers). Another product with documented antimicrobial activity is Pro-San®, previously marketed as Vegi-Clean® (www.microcideinc.com/prosan.htm). A product derived from oranges and other GRAS ingredients and claimed to have antibacterial properties is marketed under the name CitroBio for postharvest processing, use in retail misting systems, or as a produce wash for consumers (www.citrobio.com). Grapefruit seed extract (Citricidal®) is reputed to have antimicrobial properties (www.biochemresearch.com) and is being marketed as a consumer-use cleaner and disinfectant for fruits and vegetables (www.pureliquidgold.com). Other produce washes include: Veggie Wash® marketed by Beaumont Products (www.citrusmagic.com), Nature Clean Fruit & Veggie Wash (claimed to remove bacteria) (www.smallplanetinc.com, www.healthyhomeservices.ca, www.frankross.com), CleanGreens! (www.cleangreensinc.com), and Organiclean (www.organiclean.com).

In addition to these commercial products, recipes for fruit and vegetable washes can be found on the internet. Typical examples include diluted 3% hydrogen peroxide (www.wellnesstoday.com), and vinegar and 3% hydrogen peroxide sprays applied individually to produce (http://myexecpc.com/~mjstouff/articles/vinegar.html). One source suggests use of 35% hydrogen peroxide around the house, a potentially dangerous recommendation; specific uses for produce treatment call for use of 3 or 5% solutions (http://h2o2hydrogenperoxide.com/additrion.html).

17.5 CONCLUSIONS

The efficacy of conventional washing technology in reducing populations of human pathogens and other microorganisms on fresh produce surfaces is limited to 1 to 2 logs, a significant improvement compared to the unwashed produce but insufficient to ensure food safety. Incremental improvements in washing efficacy can be obtained through buffering, addition of surfactants, temperature elevation, full immersion, and washing with vigorous agitation. However, greater population reductions cannot be obtained because of the strength of microbial attachment to produce and location of attached microorganisms in inaccessible sites. Approved alternatives to chlorine may provide certain technical advantages and avoid disadvantages such as formation of toxic reaction products, but differences in antimicrobial efficacy are small. Washing agents developed for foodservice or home use may exhibit antimicrobial activity, but safe and uniform application may be problematic without the controls available for large-scale produce packing and processing applications. Microbial reduction benefits claimed by many purveyors of home-use formulations, especially those marketed via the internet, are unsubstantiated. Experimental washing agents, if found to be technically and economically feasible, or synergistic sequences or combinations of treatments may provide addition gains in efficacy over current technology, but attainment of high levels of safety such as afforded by a 5 log reduction in pathogen populations is unrealistic. Use of other technologies such as surface pasteurization or irradiation may be required to reach this level of safety.

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